

EXPERIMENTAL OBSERVATIONS ON THE PROPHYLAXIS AND TREATMENT OF SYPHILIS.

By HENRY J. NICHOLS, M.D., AND JOHN E. WALKER, M.D.

Majors, Medical Corps, U. S. Army.

(From the Laboratory Division of the Army Medical School, Washington, D. C.)

PLATE 28.

(Received for publication, November 24, 1922.)

I.

INTRODUCTION.

Experimental syphilis in the rabbit is a subject which has developed considerably in the last decade. One of the most important advances has been the conclusive demonstration by Brown and Pearce that the disease in the rabbit is essentially a general one and that the spirochetes survive for an indefinite period in animals, once infected, as proved by the transfer of the disease with the tissue of lymphatic glands. This procedure forms an excellent check on clinical and microscopic examinations of the local lesions in the testicle and scrotum. On the other hand, the recently demonstrated existence of a natural rabbit syphilis, which at first seemed to threaten to invalidate the results of experimental work, has not proved a serious complication in the kind of experiments which we have used.

Our work here reported confirms the value of the present methods of prophylaxis.

The treatment of syphilis has made great advances since the introduction of arsphenamine, but there still seems to be a school which believes that infection with *Treponema pallidum* is incurable. The evidence for this position seems to be based on relapses following inadequate treatment, on positive gland transfers in latent cases, and on the finding of spirochetes, post mortem, in old cases which have also received indifferent treatment. The possibility of a granular stage in the life cycle of the organism has also been cited in favor

of this view. The following work has been undertaken in order, if possible, to throw some light on this subject, and as far as the experimental work goes, it shows that an infection can be entirely abolished by arsphenamine.

II.

Prophylaxis.

The first preparation to be used scientifically in the prophylaxis of syphilis was the now classical 33 $\frac{1}{3}$ per cent calomel ointment of Metchnikoff. This was tested on monkeys with uniformly satisfactory results, and finally on the medical student, Maisonneuve, who offered himself for the experiment (1). The work of Metchnikoff, however, early encountered criticism, particularly from Neisser (2). Neisser's objections to calomel ointment are discussed at length by Vedder (3) in a review of the subject. Vedder calls attention to the fact that Neisser worked usually with 10 to 20 per cent calomel ointments, and that in the three instances in which he used 33 $\frac{1}{3}$ per cent as recommended by Metchnikoff two of the animals were protected against syphilis. These results are at least as good as those he obtained with other antiseptics. Neisser believed that the incorporation of the antiseptic in a fatty base interfered with its action, and he, in collaboration with Siebert, recommends a paste composed of amylum, tragacanth gelatin, and mercuric chloride.

Schereschewsky (4) advocates a quinine ointment, and has tried this out practically with troops, with "satisfactory" results. His ointment is sold under the trade name of "Duanti." It apparently contains 40 per cent quinine (5), though we have not been able to locate a statement of its exact composition by Schereschewsky. He considers that calomel ointment has little spirocheticidal action and states that it does not cause the disappearance of spirochetes within 24 hours when applied to rabbit lesions containing numerous spirochetes (6). Neither did calomel ointment interfere with the motility of spirochetes when mixed with them on a slide. Quinine ointment, on the other hand, according to the same author, caused the disappearance of spirochetes from lesions, and also stopped their motility immediately in microscopic preparations. He (7) also states that quinine ointment applied 1 or 2 hours after intercourse prevents the transmission from animal to animal of the spontaneous spirochetosis occurring in rabbits. He did not test calomel ointment in this respect, since he considered that its uselessness had been fully demonstrated by other experiments.

Schumacher (8) has recently reviewed the question of prophylaxis, and takes up in detail several of the preparations that have been recommended for this purpose. He states that the calomel ointment of Metchnikoff possesses only historical interest, since all authors have agreed that it is without effect. He says emphatically that it should be removed for all time from the list of substances to be used in the prophylaxis of syphilis. To his mind the fact that calomel is practically insoluble precludes any possible effect, owing to the absence

of Hg ions. Some of the recent criticisms of calomel ointment are surprising when it is considered that Neisser, the first author to object to it, found it at least partially successful.

Calomel ointment has been used in the prophylaxis of syphilis in both the United States Army and Navy for a number of years. The results have been eminently satisfactory, according to Vedder's summary, and as stated in the reports of the Surgeon Generals of both Services. Its value seems to have been particularly demonstrated during the recent war, as has been brought out by Young (9, 10), G. Walker (11), and many others. The fact has been recognized that its efficiency diminishes rapidly as the time after exposure increases. The ointment used in the Army has the following formula.

Calomel.....	30 parts.
Benzoinated lard.....	65 parts.
White wax.....	5 parts.

The application of the ointment is preceded by preliminary cleansing with soap and water, and the injection of protargol (1 per cent) into the urethra. Regulations require that this prophylaxis be taken as soon as possible after exposure.

As a result of experience in France during the war, Dr. Hugh Young concluded that, if possible, the prophylaxis should be simplified by omitting the preliminary washing and by using a single antiseptic which would be effective against syphilis, chancroid, and gonorrhea. Calomel ointment alone is not effective against gonorrhea, although it can be made so by the addition of 3 per cent carbolic acid and camphor as in the old Army and Navy packet (12) and the present Navy tube. There is evidence that calomel treatment is also less effective against chancroids than against chancres (11). Dr. Young suggested the use of some of the more powerful mercurial antiseptics which have been developed at the Brady Institute such as "Meroxyl" or "253." The following work was undertaken as a basic study of present methods with a view to establishing a means of comparison with other antiseptics, which are now under investigation.

A. Method.

Most of the early work was done with monkeys, but as we wished to be able to use a great number of animals, we employed rabbits.

First some preliminary experiments were done in order to work out a method for their utilization. The application of a spirochete suspension to the unbroken skin does not result in an infection. The unbroken skin of the scrotum of two animals was inoculated with a heavy suspension of spirochetes; the animals were held under observation for 90 days, gland transfers being made at the end of 45 days. None of the animals became infected. However, if the skin over both testicles is scarified as in the old vaccination method, a high percentage of takes is obtained, 100 per cent when both testicles are considered and 81 per cent of individual testicles (Table I). This method was then combined with the gland transfer method, which yielded in the controls 87.5 per cent of takes. These two procedures afford a fairly accurate method of testing prophylaxis.

The strain of *Treponema pallidum* used was the so called Nichols (13) strain which was isolated from spinal fluid in 1912 and has been carried on in rabbits ever since. That it is particularly suitable for this sort of experimental work we are not prepared to state on the basis of rigid comparative tests, but it has been largely used in other experimental work and its characteristics are well known.

1. Local Inoculation.—The testicle was drawn into the scrotum and held in such a manner that the skin over it was taut. The hair was shaved without soap, and the surface sterilized with alcohol, which was allowed to evaporate thoroughly. From fifteen to twenty superficial abrasions were then made by allowing a razor to glide lightly over the surface of an area of approximately 1 by 1.5 cm., bleeding being avoided as much as possible. About 0.05 cc. of a spirochete suspension was placed on the scarified area and rubbed lightly with the sides of the capillary pipette. This was allowed to dry and the animal was replaced in its cage. Both testicles were invariably inoculated in the same way.

At fixed intervals the animals to receive prophylaxis were removed from the cages and approximately 1 gm. of ointment was rubbed in on each side with moderate pressure by means of a wooden spatula. The control animals, of course, received no prophylactic ointment.

The spirochete suspension was made by removing a small cylinder of tissue from a chancre of the scrotum with a large bore capillary pipette, or better, by removing a testicle containing syphilitic nodules¹ and cutting out the affected portions with scissors. The latter method gave much richer spirochete suspensions, and, as will be mentioned later, saved time on account of the more rapid development of lesions. The tissue removed was ground up in a sterile mortar, and from 1 to 2 cc. of salt solution were added. The addition of a small

¹ All operations were performed under ether anesthesia.

amount of sand or a few bits of broken glass aided in the grinding. It is inadvisable to add citrate solution, as this interferes with the motility of the organisms and distorts them somewhat. It does not interfere with their viability, however.

As Tables I and III show, the animals were inoculated in four groups at different times, and hence of necessity with different spirochete suspensions. The number of spirochetes in each suspension was estimated by counting microscopic fields. Since the suspensions varied in the number of spirochetes, an opportunity was afforded of determining whether the strength of the suspension affected the incubation period of the disease.

Groups 1 and 2 were inoculated from suspensions containing three to five spirochetes per field, Group 3 from a suspension containing one to three spirochetes per field, and Group 4 from a rich suspension obtained from testicular material containing six to thirteen spirochetes per field. Three controls were used with Group 1, one control with Group 2, and two controls each with Groups 3 and 4. The controls for Group 1 were positive in from 33 to 36 days, for Group 2 in 32 days, for Group 3 in 35 to 49 days, and for Group 4 in 16 to 17 days. Hence it seems that the rich suspension used for Group 4 shortened considerably the incubation time of the disease.

One of the controls for each group was invariably inoculated at the end of the experiment; that is, after all the other animals had been inoculated. This was done in order to be absolutely certain that the organisms were not injured by being kept at room temperature while the animals were being inoculated. All these controls were positive, showing that the organisms apparently were in no way injured. In Group 4 a period of 1 hour and 35 minutes elapsed between the inoculation of the first animals and that of the last control. Moreover, the organisms at the end of this time were still actively motile.

All animals, with the exceptions noted in the tables, were kept under observation for a period of at least 90 days. They were examined microscopically once a week, or oftener. Microscopic examinations with the dark field were made of animals that showed at the site of inoculation or of gland transfer any edema or thickening of the skin or subcutaneous tissue. The microscopic examinations were made by grasping the suspicious area between the blades of a pair of thumb forceps, and arranging the tissue in such a way that the blood was forced out, when the area appeared pale and distended by the subcutaneous fluid. The distended skin was then punctured and the drop of clear serum oozing out was collected by touching it with the surface of a cover-slip. This was immediately examined under the dark-field microscope. No animal was considered positive until proved so microscopically. The date of such proof is taken as the date of becoming positive. In practise it always coincided

with the first appearance of a characteristic initial lesion, which usually rapidly developed into a definite chancre.

The initial lesions consisted of a circumscribed area of from $\frac{1}{4}$ to $\frac{1}{3}$ cm. in diameter, showing slight edema, redness, and some thickening of the skin. The blood vessels leading into them were usually dilated. When presenting this characteristic appearance they never failed to be positive microscopically. In about a week they usually developed into pale button-like areas of induration, followed by ulceration and progressive enlargement of the chancre until it became knob-like and of a diameter of 1.5 to 2 cm. or more. As noted, however, some of the lesions did not progress this far.

A few of the animals developed a diffuse non-specific inflammation of the skin of the scrotum. The thickening and edema embraced not only the inoculated area, but practically the whole scrotum. The edema was sometimes marked. Despite the fact that the appearance was not at all characteristic, all animals showing it were repeatedly examined microscopically, with negative results. This inflammation was attributed to trauma from the shavings in the bottom of the cage, especially since the hair of the scrotum had been shaved away.

The size of the inguinal lymph glands was noted on all examinations. Their reaction was inconstant, as in some animals they were markedly enlarged, and in others with similar local lesions they were not palpable.

2. Gland Transfers.—In transferring glands from one animal to another, we varied somewhat the technique of Pearce and Brown (14). They removed the gland, ground it up in a mortar, and injected it into the testicular substance with a syringe. The variation we made consisted of removing the gland and placing it *in toto* in the subcutaneous tissue of the scrotum through a small opening made in the skin. The opening was then closed with collodium. This method left the gland in a superficial position, where any changes in it or in the surrounding tissue could be readily followed and easily examined microscopically. One of the inguinal glands was always used, being removed under ether anesthesia. Few suppurations occurred, and even when they did there was apparently no interference with the subsequent development of chancres. The gland transfers were made only after the controls had become positive, 28 to 72 days from the date of inoculation.

The animals receiving glands from cases clinically negative were without exception kept under observation for at least 90 days. As shown in Table III, they were all negative, and require no further comment.

B. Results.

Table I shows the results obtained with eight animals inoculated as controls, which received no prophylactic ointment. They were

TABLE I.

Control Animals. Rabbits Inoculated with Treponema pallidum by Scarification of the Skin of the Scrotum; No Prophylactic Ointment Applied.

Experiment No.	Rabbit No.	Group.	Result.		Day testicle became positive for spirochetes.	Remarks and description of lesions.
			Right side.	Left side.		
1	1	1	+	+	36	Bilateral indurated and ulcerated chancres 1.5 cm. in diameter. Lesions were regressing when animal was discarded on 90th day.
2	2	1	+	+	36	"Blank" ointment applied 1 hr. after inoculation. Bilateral indurated and ulcerated chancres. Complete disappearance of lesions by the 88th day.
3	3	1	-	+	33	Left side only positive; small initial lesion. Small suspicious lesion on right side, not positive microscopically. Animal died on 33rd day from pneumonia.
4	4	2	-	+	32	Left side only positive; small indurated chancre 1 cm. in diameter, disappearing completely by 67th day. Gland transfer made on 68th day positive.
5	5	3	+	-	35	Right side only positive; indurated ulcerated chancre 1 cm. in diameter. No lesion on left side (Fig. 1). Animal died of pneumonia on 58th day.
6	6	3	+	+	49	Small bilateral areas of localized edema and slight induration, disappearing in a few days. Gland transfer made on 41st day positive.
7	7	4	+	+	17	Large bilateral indurated and ulcerated chancres, 2.5 cm. in diameter. Chancres were regressing when animal was discarded on 90th day.
8	8	4	+	+	16	Large bilateral indurated and ulcerated chancres, 1.5 cm. in diameter. Animal sacrificed on 55th day for other experiments.

Of eight controls, 100 per cent were positive on one side or the other. 81 per cent of the individual testicles were positive.

100 per cent positive for syphilis in one testicle or the other and 81 per cent of the individual testicles were positive. One animal (No. 2) received an application of a "blank" ointment, consisting of a mixture

of equal parts of lanolin and vaseline. The ointment was applied 1 hour after inoculation. This was done to determine whether the base of the ointment alone has any effect, particularly since some authors have raised the question of whether the fatty base in itself might not have an inhibiting effect. In this experiment the base alone had apparently no effect, since the animal developed syphilis.

TABLE II.
Control Gland Transfers. Gland Transfers from Known Syphilitic Animals.

Experiment No.	No. of original rabbit.	Day of gland transfer.	No. of rabbit receiving gland.	Result of gland transfer.	Day gland transfer became positive for spirochetes.	Remarks and description of lesions in animals receiving glands.
1	6 (Table I).	41	15	+	41	Large chancre, 2 cm. in diameter, indurated and ulcerated. Lesions still present when animal was discarded on 90th day.
2	9	41	16	+	20	Large chancre.
3	4 (Table I).	68	17	+	77	Small area of edema and induration at site of gland transfer, disappearing in about 7 days.
4	10	105	18 (left side).	+	32	Large chancre.
5	11	170	18 (right ").	+	36	" "
6	12	231	20	+	47	" "
7	13	261	21	+	56	" "
8	14 (Table III).	30	22	—	—	No lesion after 90 days.

With gland transfers 30 to 261 days after inoculation, of eight animals, seven, or 87.5 per cent, were positive.

Rabbits 3 and 6 developed only small lesions. Both these animals, however, began to lose weight soon after beginning the experiment; one died on the 33rd day, and the other on the 49th day, after developing pneumonia that ran a rather chronic course. The pneumonia was due to an organism of the hemorrhagic septicemia group. Loss of weight in an animal showing lesions was usually accompanied by disappearance or regression of the lesions. The healthier the

animal, the more characteristic was the lesion. Hence, healthy stock is of great importance for the success of the inoculations as is a rich spirochete suspension.

TABLE III.
Animals Treated with 30 Per Cent Calomel Ointment at Varying Intervals after Inoculation.

Experiment No.	Rabbit No.	Group.	Interval before application of ointment.	Result.	Day of transfer.	Result of transfer.	Remarks.
			hrs.				
1	23	4	1	—	29	—	Original animal died of pneumonia on 35th day.
2	24	1	1	—	53	—	
3	25	2	2	—	44	—	
4	26	3	2	—	39	—	Original animal died of pneumonia on 39th day.
5	27	4	3	—	29	—	
6	28	3	3	—	41	—	
7	29	3	4	—	41	—	
8	30	3	4	—	78	—	
9	31	4	4	—	27	—	Original animal died of pneumonia on 27th day.
10	32	4	5	—	29	—	
11	33	4	5	—	37	—	
12	34	4	6	—	37	—	
13	35	4	6	—	28	—	
14	36	4	7	—	30	—	Original animal died of pneumonia on 63rd day.
15	37	4	7	—	37	—	
16	14	4	8	+	30	—	Left side only positive; small area of localized edema and slight thickening, rapidly disappearing. Right side showed slightly suspicious area, not positive microscopically.
				(36 days).			

All transfers were observed for 90 days.

Table II shows the results of eight control gland transfers from positive animals. Seven, or 87.5 per cent, were positive. The percentage is less than that of Brown and Pearce, but is sufficiently high to be of value when taken with the results of Table I.

Table III shows that in fifteen instances the calomel prophylaxis was successful when practised for periods up to 8 hours after inoculation. Neither the original animal nor the transfer showed the slightest evidence of infection. At 8 hours, the only animal treated developed a chancre. These results were clear-cut and convincing, especially when compared with the results in the controls, in the animal treated with blank ointment, and in the animals treated with other antiseptics.

C. Influence of Base Ointment.

Metchnikoff's original ointment was composed of ten parts of calomel and twenty parts of lanolin. Vedder (3), in reviewing the subject, likewise concluded that lanolin was the proper base for the calomel. As stated above, the Army has used for a number of years a base composed of lard and white wax.

We tested calomel in a base of lard and white wax, and also in a base of lanolin and vaseline. In Table III the animals are grouped according to the hour at which they were treated. Of each pair dealt with at a given hour one was treated with a lard and wax, and one with a lanolin and vaseline base, except at 4 hours, when one received the lard and wax base, and two lanolin and vaseline, and at 8 hours, when the single animal was treated with a lard and wax base. The result shows that, if there is any difference dependent on the base, it must be slight.

D. Death of Animals Due to Prophylaxis.

An example of the absorption of mercury and the sensitiveness of the rabbit to mercury occurred in five animals which died of mercurial nephritis following prophylaxis with calomel ointment. The animals were given an application on the scrotum of about 5 gm. of calomel ointment and died in 5 to 15 days. The only lesion found post mortem was in the kidney. The pathological report on one of these kidneys is as follows:²

"The kidneys show an advanced granular degeneration of all parenchymatous structures with fatty degeneration and necrosis of the type cells. Extensive deposits of lime salts in the convoluted tubules and Henle's loops typical of acute mercurial poisoning in man are found everywhere throughout the cortex."

² This report was kindly made by Major G. R. Callender, of the Army Medical Museum.

The animals dying were replaced by animals with which only 2 gm. of calomel ointment were used, and no further difficulty was experienced. In order to be absolutely certain that the deaths were due to calomel ointment, six normal animals were treated with from 5 to 15 gm. of calomel ointment. These animals all died with typical kidney changes in from 5 to 7 days. There was no difference between calomel in a lard base and calomel in a lanolin and vaseline base in this respect. These six animals were maintained for 48 hours in narrow inoculation boxes, which precluded the possibility of ingestion by licking.

It is surprising that an inert insoluble substance such as calomel should be absorbed so readily through the skin. The danger is thus shown of attempting to predict the physiological, and perhaps also the bactericidal, properties of a substance from its physical properties. Our observation in this particular confirms the previous work of Schamberg and his coworkers (15) who showed that inunctions of calomel ointment caused the death of rabbits. One of their animals died after two inunctions, each of 1.3 gm. of 50 per cent calomel ointment, given with a 5 day interval. Their animals, however, did not show the marked kidney lesions that were present in our animals that died following prophylaxis. As a result of their work they urged that inunctions of calomel ointment be used in the treatment of syphilis. Cole and Littman (16) tried this out clinically with results that were not satisfactory, though Schamberg, in discussing their paper, suggested that larger amounts of the ointment should be used.

III.

Treatment.

A. Method.

The problem was to test by the gland transfer method the sterilizing effect of arsphenamine and neoarsphenamine on rabbits infected with *Treponema pallidum*. For this purpose animals were selected which had had typical inoculation chancres and which had been infected long enough for a general infection to become thoroughly established. In other words, they were either latent or old cases in the human sense, with disease lasting from 3 to 6 months. They were treated with a different number of doses as indicated in Table IV and at

intervals from 28 to 61 days after treatment a gland was transferred. The original animal was observed during 90 days for relapse and the transfer animal was watched for 90 days for evidence of infection around the site of inoculation. The drugs used were of the German variety dating to before the war, but may be supposed to differ from other brands only in degree of effectiveness.

B. Result.

Of six animals treated in this way, not one gave evidence of infection, even after a single intravenous dose (0.01 gm. per kilo of "606," 0.015 gm. per kilo of "914"). It is known (Brown and Pearce) that rabbits show clinical relapses after subcurative doses. It may

TABLE IV.
Results of Treatment.

Experiment No.	Rabbit No.	Treatment.	Time after infection. <i>days</i>	No. of animal receiving gland.	Time after treatment. <i>days</i>	Result of gland transfer.	Remarks.
1	38	One dose of neoarsphenamine, 0.015 gm. per kilo.	130	44	28	—*	Latent case. Two large chancres healed before treatment. No relapse within 97 days.
2	39	One dose of arsphenamine, 0.01 gm. per kilo.	130	45	28	—*	Old case still active at time of treatment. No relapse within 97 days.
3	40	Two doses of neoarsphenamine.	125	46	29	—*	Old case, still active at time of treatment. No relapse within 90 days.
4	41	Two doses of arsphenamine.	174	47	29	—*	Old case, still active at time of treatment. No relapse within 90 days.
5	42	Four doses of arsphenamine.	110	48	48	—*	Old case, still active at time of treatment. No relapse within 104 days.
6	43	Four doses of arsphenamine.	125	49	61	—†	Transfer from No. 49 to No. 50 negative after 91 days.

* Observed 90 days.

† Observed 28 days.

be objected that 28 days was too short a time for the spirochetes to recover from the effects of treatment and transfer. It is true that clinical relapses occur in the 1st and 2nd months and 4 weeks seemed a fair time for some of the animals, but two of them were held for 8 and 9 weeks before transfer. This surely gives sufficient time for infection to develop. We do not wish to argue from this experiment that a single full intravenous dose is curative in man any more than we wish to warn against mercurial poisoning from prophylaxis. The rabbit has its own characteristics and responds to drugs in its own way. What we do wish to emphasize is that by the most rigid tests available, syphilis has been abolished by arsphenamine in a mammal. From this it may be concluded that by sufficient treatment the same can be accomplished in man. By cure is meant the death of the last spirochete (17).

IV.

Venereal Spirochetosis of Rabbits.

Noguchi (18) has recently called attention to the occurrence in rabbits of a natural venereal spirochetosis. This disease was previously described by certain European observers, notably Arzt and Kerl (19) and more recently by Schereschewsky (7) and Klarenbeek (20). The work of these and other authors is reviewed by Noguchi. The disease consists of ulcerative papular lesions covered with crusts, principally on the vulva or on the sheath of the penis. Examination of serum expressed from the lesions reveals spirochetes, usually in large numbers, similar, morphologically, to *Treponema pallidum*. Noguchi suggests the name *Treponema cuniculi* for these organisms. The disease is chronic, and persists over an indefinite period of time, with occasional inflammatory exacerbations, during which the area involved is reddened and swollen. These inflammatory exacerbations are easily brought on by manipulation of the parts. The disease occurs under natural conditions and Noguchi found it in five out of fifty animals regarded as normal.

During the course of the present work every possible precaution was taken to prevent any complication of the experiments with this condition. Careful and repeated examination of the stock revealed no lesions. All incoming animals were examined, and they were not

used in experiments until they had been under observation 2 weeks or more. The repeated examination of the animals revealed no lesions resembling those described as due to *Treponema cuniculi*. In order that we might have a close personal experience with the condition and in view of our inability to find it among our animals, we requested Dr. Noguchi to send us one of his animals with lesions of the disease. He kindly sent us a female that had had lesions on the vulva for months. The lesions showed numerous spirochetes with the tendency to form the entangled masses of stellate arrangement noted by Dr. Noguchi. The organisms as a rule seem longer than *pallidum* and somewhat more slender, but in some instances it is impossible morphologically to distinguish the two kinds.

We inoculated the skin of the scrotum of a normal rabbit with serum containing spirochetes from this female, in the same manner in which the animals in the prophylaxis experiments were inoculated. When this animal was examined 5 days later the site of inoculation was slightly reddened and edematous. Examination of serum from the lesion showed an occasional spirochete. Hence the inoculation was positive after an incubation period of only 5 days. The lesion 16 days after inoculation was a slightly raised area about 0.75 cm. in diameter, of much the appearance of an early chancre (Fig. 2). However, it remained stationary, except for becoming slightly more diffuse, for 14 weeks, when it acquired the character of a very superficial non-indurated ulceration. When last observed, 20 weeks after inoculation, the lesion was still present and positive for spirochetes. The persistence of the lesion was in marked contrast to syphilitic lesions which either disappeared completely in a short time, or else went on to the formation of large typical chancres.

A lymph gland was removed from this animal 7 days after inoculation and implanted in the scrotum of a normal animal. A second gland was removed on the 43rd day and transplanted in a second animal. Both these animals receiving gland transplants remained free of any evidence of the disease during the observation period of 90 days. Noguchi was similarly unable to transmit the infection by testicular injection of lymph gland emulsions from ten animals infected with *Treponema cuniculi*. We also transferred a gland from the female sent us by Dr. Noguchi with negative result. Hence,

both in the character and the course of the original lesion and in the failure of gland transfer, there is a marked difference from syphilis.

Noguchi attempted to transmit the disease by mating infected animals with normal animals. He was successful in one instance. Schereschewsky (7), however, was able to transmit the disease sexually with great ease, and, as mentioned above, utilized this method of transmission for testing the value of his prophylactic ointment containing quinine, "Duanti."

The Wassermann reactions of two animals infected with *Treponema cuniculi* were negative, as found by Dr. Noguchi. However, of thirteen of our animals known to be infected with syphilis, only four had positive Wassermann reactions. Hence, the Wassermann reaction seems to be of little aid in differentiating the two conditions.

We do not believe that this disease has been mistaken in the present work. The material for inoculation was invariably obtained from large indurated chancres and the lesions considered syphilitic by us developed at the site of inoculation.

However, lesions due to *Treponema cuniculi* did occur later in two of the rabbits, No. 51 (used in other experiments; Fig. 3) and No. 52 (Fig. 4), one with syphilitic lesions and the other without.

On the 55th day, after inoculation with syphilitic virus, Rabbit 51 had large bilateral chancres. At this time, during the routine examination of the genitalia, it was noted that there were small superficial scaly lesions on the sheath of the penis. Examination of these revealed a few spirochetes. Transfers were made to other rabbits from the chancres and from the lesion of the sheath. The material from the chancre produced a typical large chancre. Serum expressed from the lesion on the penis and subcutaneously injected in the scrotum of a second animal produced an initial lesion after 34 days that was positive for spirochetes. This lesion proceeded to slight superficial ulceration, without induration, and persisted without change until the animal died on the 77th day. Spirochetes were constantly found in it. The difference in these two transfers from the same animal was striking. A transfer from Rabbit 52 to a normal animal also produced a small elevated lesion, proceeding to superficial ulceration, which persisted without showing any induration.

In order to determine whether *Treponema pallidum* would produce a lesion on the sheath of the penis apt to be mistaken for *Treponema cuniculi*, a rabbit (No. 53) was inoculated with syphilitic virus by scarifying the sheath.

41 days after inoculation the mucous membrane of the sheath was swollen and ulcerated at the site of inoculation. Examination for treponemata was positive. The lesion rapidly advanced, and induration of the sheath was marked. To the touch it was hard and cartilaginous. There was no tendency to scaling as in lesions due to *Treponema cuniculi*. The appearance 65 days after inoculation, at which time, however, the lesion had begun to subside, is shown in Fig. 5, for comparison with the *cuniculi* lesion in Fig. 4.

We have, then, a disease of rabbits due to a spirochete often indistinguishable morphologically from the organism of human syphilis. Rabbits infected with it do not have positive Wassermann reactions, while rabbits with human syphilis sometimes have positive reactions. The natural disease takes the form of ulcerated papules in the region of the genitalia. Inoculation of the material into the scrotum may produce lesions somewhat resembling early chancres, as in Dr. Noguchi's rabbit and our rabbit mentioned above. These lesions, however, persist, without either disappearing or advancing (except for superficial ulceration), in contrast to those of syphilis. In syphilis, when the lesion does not go on to the formation of a typical chancre, it immediately disappears, as exemplified in Rabbits 6 (Table I) and 14 (Table III).

Spontaneous lesions of the rabbit disease apparently do not occur on the scrotum, and, when experimentally induced there, differ from the characteristic button-like chancre of the strain of *pallidum* used in the present work. We are of the opinion, therefore, that this natural disease is not a more serious complication in work with lesions of the scrotum and testicle than the disturbance caused by other acid-fast organisms in work on experimental tuberculosis. Experimental work on generalized syphilis, however, and especially on sexual transmission, should be considered with this disease in mind. In its effect on the rabbit, it is more comparable with venereal infection with the organisms of Vincent's angina in man than with the disease due to *Treponema pallidum*.

V.

SUMMARY.

1. By inoculating the scarified surface of both sides of the scrotum of rabbits with suspensions of *Treponema pallidum*, 100 per cent of

infections were obtained on one side or the other. Infection through the unbroken skin could not be produced.

2. By gland transfers from animals with positive local inoculations, 87.5 per cent of takes were produced.

3. These two methods were used to test the prophylactic value of 30 per cent calomel ointment. (a) Calomel ointment proved efficacious up to 8 hours after inoculation with syphilis. (b) No marked difference appeared between the action of calomel in a base of lanolin and vaseline and in a base of benzoinated lard and wax. (c) Death from mercurial poisoning was produced in rabbits by a single application of a large amount of calomel ointment.

4. The method of gland transfers was used to test the sterilizing effect of arsphenamine and neoarsphenamine on old infections in the rabbit. The infection was completely abolished in every instance, whether by one, two, or four intravenous doses.

5. Natural spirochetosis of rabbits need not be a serious complicating factor in work on syphilis in rabbits, for the following reasons. (a) In natural spirochetosis, the lesions occur on the penis and not on the scrotum. Gland transfers are negative. (b) A scrotal lesion can be produced by inoculation, but it can be distinguished from that of *Treponema pallidum* infection by its course. (c) In studies of generalized syphilis supposed to involve the genitalia, and in sexual transmission experiments, *Treponema cuniculi* may be a serious complicating factor.

BIBLIOGRAPHY.

1. Metchnikoff, E., and Roux, E., *Bull. Acad. méd.*, 1906, lv, series 3, 554.
2. Neisser, A., *Verhandl. deutsch. dermat. Ges.*, 1906, ix, 83.
3. Vedder, E. B., Syphilis and public health, Philadelphia and New York, 1918.
4. Schereschewsky, J., *Berl. klin. Woch.*, 1919, lvi, 752.
5. Control of venereal disease, *Lancet*, 1922, i, 292.
6. Schereschewsky, J., and Worms, W., *Deutsch. med. Woch.*, 1921, xlvii, 176.
7. Schereschewsky, J., *Berl. klin. Woch.*, 1920, lvii, 1142.
8. Schumacher, J., *Deutsch. med. Woch.*, 1921, xlvii, 626.
9. Young, H. H., *J. Am. Med. Assn.*, 1919, lxxiii, 1668.
10. Young, H. H., *Mil. Surg.*, 1921, xlviii, 213.
11. Walker, G., *J. Am. Med. Assn.*, 1922, lxxviii, 1510.
12. Russell, F. F., and Nichols, H. J., *Mil. Surg.*, 1912, xxxi, 214.
13. Nichols, H. J., *J. Exp. Med.*, 1914, xix, 362.

14. Pearce, L., and Brown, W. H., *J. Exp. Med.*, 1922, xxxv, 39.
15. Schamberg, J. F., Kolmer, J. A., Raiziss, G. W., and Gavron, J. L., *J. Am. Med. Assn.*, 1918, lxx, 142.
16. Cole, H. N., and Littman, S., *J. Am. Med. Assn.*, 1919, lxxiii, 1409.
17. Craig, C. F., and Nichols, H. J., Studies of syphilis, *U. S. War Dept., Office of Surgeon General, Bull. 3*, 1913, 129.
18. Noguchi, H., *J. Exp. Med.*, 1922, xxxv, 391.
19. Arzt, L., and Kerl, W., *Wien. klin. Woch.*, 1914, xxvii, 1053.
20. Klarenbeck, A., *Ann. Inst. Pasteur*, 1921, xxxv, 326.

EXPLANATION OF PLATE 28.

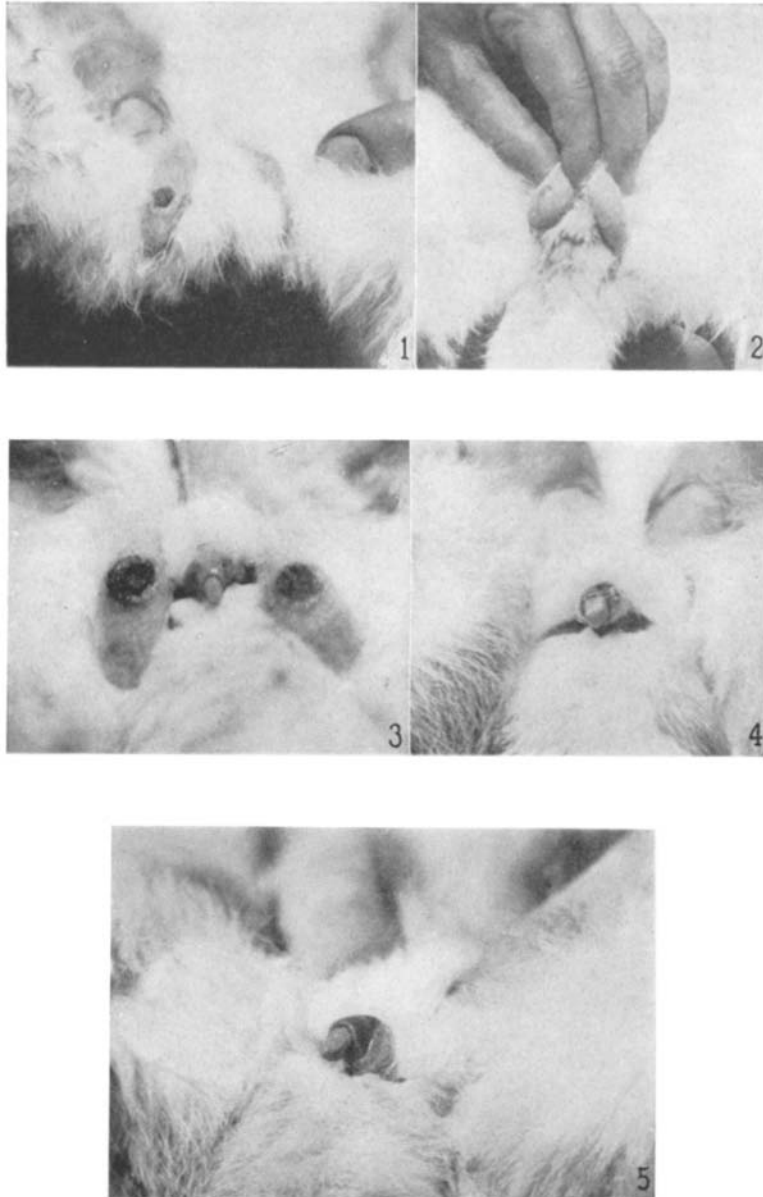
FIG. 1. Rabbit 5; control; 54 days after inoculation. Chancre on the right side. There was no lesion on the left.

FIG. 2. Rabbit 54. Lesion due to *Treponema cuniculi* on the right side of the scrotum 16 days after inoculation.

FIG. 3. Rabbit 51; 65 days after inoculation. Large bilateral chancres. The sheath of the penis has been drawn back. Lesions, positive for *Treponema cuniculi*, are barely visible in the photograph, on each side of the sheath of the penis just where the mucous membrane joins the skin covered with hair.

FIG. 4. Rabbit 52. Scaly lesions of *Treponema cuniculi* on the sheath of the penis.

FIG. 5. Rabbit 53; 65 days after inoculation of the sheath with *Treponema pallidum*. Chancre of the sheath of the penis.



(Nichols and Walker: Prophylaxis and treatment of syphilis.)